PATENT
Docket No. 246152002603

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Marian Christopher

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Robert VAN GORCOM et al.

Serial No.: (not yet assigned)

(CON of 09/233,510)

Filing Date: January 20, 1999

For: CLONING AND EXPRESSION OF MICROBIAL PHYTASE

Examiner: Unassigned

Group Art Unit: Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the application as follows:

In the Specification:

On page 1, before the paragraph entitled Technical Field, please insert the following paragraph:

--This patent application is a continuation of and claims priority to U.S. Application Serial No. 09/233,510, filed 20 January 1999, which is a continuation of Serial No. 08/419,448

filed 10 April 1995, issued as U.S. Patent No. 5,863,533 on 29 January 1999, which is a divisional of Serial No. 08/151,574 filed 12 November 1993, issued as U.S. Pat. No. 5,436,156 on 25 July 1995, which is a continuation of Serial No. 07,688,578 filed 24 May 1991, which is a U.S. national phase application of PCT/NL90/00140 filed on 27 September 1990, which are incorporated herein by reference.--

On page 9, line 12, after "Figure 1. A." please insert --(SEQ ID NO:1), (SEQ ID NO:2), (SEQ ID NO:6)--.

On page 9, line 24, after "B." please insert --(SEQ ID NO:4), (SEQ ID NO:5), (SEQ ID NO:7), (SEQ ID NO:8), (SEQ ID NO:9)--.

On page 9, line 31, after "C." please insert --(SEQ ID NO:3)--.

On page 10, line 1, after "Figure 2. A." please insert --(SEQ ID NO:10), (SEQ ID NO:14), (SEQ ID NO:15), (SEQ ID NO:16), (SEQ ID NO:17), (SEQ ID NO:18), (SEQ ID NO:19), (SEQ ID NO:20), (SEQ ID NO:21), (SEQ ID NO:22), (SEQ ID NO:23), and (SEQ ID NO:24)--.

On page 10, line 3, after "Figure 2.B." please insert --(SEQ ID NO:11), (SEQ ID NO:12) (SEQ ID NO:25), (SEQ ID NO:26), or (SEQ ID NO:27)--.

On page 10, line 5, after "Figure 3." please insert --(SEQ ID NO:13), (SEQ ID NO:28); (SEQ ID NO:29) and (SEQ ID NO:30)--.

On page 10, line 22, after "Figure 6." please insert --(SEQ ID NO:31) and (SEQ ID NO:32)--.

On page 11, line 1, after "Figure 8." please insert --(SEQ ID NO:33)--.

On page 40, line 14, after the sequence please insert --(SEQ ID NO:34)--.

On page 40, line 16, after the sequence please insert --(SEQ ID NO:35)--.

On page 40, line 17, after the sequence please insert --(SEQ ID NO:36)--.

On page 40, line 18, after the sequence please insert --(SEQ ID NO:37)--.

On page 46, line 30, after the sequence please insert --(SEQ ID NO:38)--.

On page 46, line 31, after the sequence please insert --(SEQ ID NO:39)--.

On page 47, line 8, after the sequence please insert --(SEQ ID NO:40)--.

On page 47, line 11, after the sequence please insert --(SEQ ID NO:41)--.

On page 47, line 13, after the sequence please insert --(SEQ ID NO:42)--.

On page 47, line 15, after the sequence please insert --(SEQ ID NO:43)--.

On page 48, lines 12 and 13, after the sequence please insert --(SEQ ID NO:44)--.

On page 48, line 34, after the sequence please insert --(SEQ ID NO:45)--.

On page 48, line 36, after the sequence please insert --(SEQ ID NO:46)--.

On page 49, line 24, after the sequence please insert --(SEQ ID NO:47)--.

On page 49, line 26, after the sequence please insert --(SEQ ID NO:48)--.

On page 54, line 11, after the sequence please insert --(SEQ ID NO:49)--.

On page 54, line 18, after the sequence please insert --(SEQ ID NO:50)--.

On page 54, line 36, after the sequence please insert --(SEQ ID NO:51)--.

On page 54, line 37, after the sequence please insert --(SEQ ID NO:52)--.

In the Claims:

Please cancel Claims 1-31 without prejudice or disclaimer, and add the following claims:

32. A purified and isolated DNA molecule which:

a) encodes a fungal phytase which catalyzes the liberation of inorganic phosphorus from myoinositol hexakis-phosphate; and

b) encodes a phytase that is encoded by a nucleotide sequence that is selected from the group of nucleotide sequences consisting of:

a nucleotide sequence hybridizing with a probe selected from SEQ ID NO:25, SEQ ID NO:26 or SEQ ID NO:27; and

a nucleotide sequence hybridizing to a cDNA probe comprising nucleotides 210-1715 of SEQ ID NO:31 under conditions of low stringency (6xSSC; 50°C. overnight), or a nucleotide sequence derived from said nucleotide sequence by degeneration of the genetic code.

33. A purified and isolated DNA molecule which is derived from the DNA molecule according to claim 32 by degeneration of the genetic code.

34. A recombinant expression system which is useful, when contained in a host cell, for expressing a nucleotide sequence encoding a fungal phytase which catalyzes the liberation of inorganic phosphate from myoinositol hexakis-phosphate, and

wherein said phytase is encoded by a nucleotide sequence that is selected from the group of nucleotide sequences consisting of:

a nucleotide sequence hybridizing with a probe selected from SEQ ID NO:25, SEQ ID NO:26 or SEQ ID NO:27;

said expression system comprising a nucleotide sequence encoding said phytase, or a nucleotide sequence derived therefrom by degeneration of the genetic code, operably linked to control sequences compatible with said host cell.

35. The expression system of claim 34, wherein said nucleotide sequence encoding said protein further includes a sequence encoding a secretory leader sequence operably linked to said protein.

36. The expression system of claim 35, wherein said leader sequence comprises the 18-amino acid AG leader sequence.

37. The expression system of claim 34 wherein said control sequence includes an AG promoter.

38. A recombinant vector comprising the expression system of claim 37.

39. A recombinant microbial host cell comprising the expression system of claim 34.

40. The cell of claim 34 which is a bacterial, yeast or fungal cell.

41. The cell of claim 40 which is of a genus selected from the group consisting of *Aspergillus, Trichoderma, Penicillium, Mucor, Bacillus, Kluyveromyces and Saccharomyces.*

42. The cell of claim 41, which is of a species selected from the group consisting of *Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Trichoderma reesei, Mucor miehei, Kluyveromyces lactis, Saccharomyces cerevisiae, Bacillus subtilis* and *Bacillus licheniformis.*

43. A method to express a nucleotide sequence encoding a fungal phytase which phytase catalyzes the liberation of inorganic phosphate from myoinositol hexakis-phosphate, which method comprises

(a) culturing the cells of claim 39 under conditions wherein said phytase-encoding nucleotide sequence is expressed to produce said phytase, and

(b) recovering said phytase produced from said culture.

44. A method to identify nucleic acid molecules that encode compounds having phytase activity which method comprises

subjecting a candidate nucleic acid molecule to an assay to assess its ability to hybridize with a probe selected from SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27, and

subjecting a compound encoded by a nucleic acid molecule that hybridizes to said probe to an assay that measures phytase activity, wherein a nucleic acid molecule that encodes a compound that has phytase activity is identified.

45. The method defined in claim 44 wherein the assay that measures phytase activity comprises determining whether the compound encoded by a nucleic acid molecule catalyzes the liberation of inorganic phosphorous from myoinositol hexakis-phosphate

46. The method defined in claim 44, wherein the nucleic acid molecule is obtained from a fungal source.

47. A recombinant expression system which is useful, when contained in a host cell, for expressing a nucleic acid molecule encoding a phytase, the expression system comprising a nucleic acid molecule identified according to the method of claim 44, or a nucleic acid molecule derived therefrom by degeneration of the genetic code, operably linked to control sequences compatible with the host cell.

REMARKS

New claims 31-43 include the subject matter deleted from claims 43 and 46 *et seq*. in the response to the final office action of May 9, 2000 of the parent application having Serial No. 09/233,510, which response was dated February 14, 2001. Support for new claims 44-46 is found in prior claim 43 as well on page 7, lines 21-30 and page 15, lines 16-30. Support for claim 47 is found, for example, on page 16, lines 6-22.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

No new matter has been added and consideration of these claims is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 246152002603.

Respectfully submitted,

Dated: February 19, 2002 By: [signature]

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 1, before the paragraph entitled Technical Field, the following paragraph has been inserted:

--This patent application is a continuation of and claims priority to U.S. Application Serial No. 09/233,510, filed 20 January 1999, which is a continuation of Serial No. 08/419,448 filed 10 April 1995, issued as U.S. Patent No. 5,863,533 on 29 January 1999, which is a divisional of serial no. 08/151,574 filed 12 November 1993, issued as U.S. Pat. No. 5,436,156 on 25 July 1995, which is a continuation of Serial No. 07,688,578 filed 24 May 1991, which is a U.S. national phase application of PCT/NL90/00140 filed on 27 September 1990, which are incorporated herein by reference.--

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On page 54, line 36, after the sequence please insert --(SEQ ID NO:51)--.

On page 54, line 37, after the sequence please insert --(SEQ ID NO:52)--.

In the Claims:

The following new claims have been added:

32. (New) A purified and isolated DNA molecule which:

a) encodes a fungal phytase which catalyzes the liberation of inorganic phosphorus from myoinositol hexakis-phosphate; and

b) encodes a phytase that is encoded by a nucleotide sequence that is selected from the group of nucleotide sequences consisting of:

a nucleotide sequence hybridizing with a probe selected from SEQ ID NO:25, SEQ ID NO:26 or SEQ ID NO:27; and

a nucleotide sequence hybridizing to a cDNA probe comprising nucleotides 210-1715 of SEQ ID NO:31 under conditions of low stringency (6xSSC; 50°C. overnight), or a nucleotide sequence derived from said nucleotide sequence by degeneration of the genetic code.

33. (New) A purified and isolated DNA molecule which is derived from the DNA molecule according to claim 32 by degeneration of the genetic code.

34. (New) A recombinant expression system which is useful, when contained in a host cell, for expressing a nucleotide sequence encoding a fungal phytase which catalyzes the liberation of inorganic phosphate from myoinositol hexakis-phosphate, and

wherein said phytase is encoded by a nucleotide sequence that is selected from the group of nucleotide sequences consisting of:

a nucleotide sequence hybridizing with a probe selected from SEQ ID NO:25, SEQ ID NO:26 or SEQ ID NO:27;

said expression system comprising a nucleotide sequence encoding said phytase, or a nucleotide sequence derived therefrom by degeneration of the genetic code, operably linked to control sequences compatible with said host cell.

35. (New) The expression system of claim 34, wherein said nucleotide sequence encoding said protein further includes a sequence encoding a secretory leader sequence operably linked to said protein.

36. (New) The expression system of claim 35, wherein said leader sequence comprises the 18-amino acid AG leader sequence.

37. (New) The expression system of claim 34 wherein said control sequence includes an AG promoter.

38. (New) A recombinant vector comprising the expression system of claim 37.

39. (New) A recombinant microbial host cell comprising the expression system of claim 34.

40. (New) The cell of claim 34 which is a bacterial, yeast or fungal cell.

41. (New) The cell of claim 40 which is of a genus selected from the group consisting of *Aspergillus*, *Trichoderma*, *Penicillium*, *Mucor*, *Bacillus*, *Kluyveromyces and Saccharomyces*.

42. (New) The cell of claim 41, which is of a species selected from the group consisting of *Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Trichoderma reesei, Mucor miehei, Kluyveromyces lactis, Saccharomyces cerevisiae, Bacillus subtilis* and *Bacillus licheniformis*.

43. (New) A method to express a nucleotide sequence encoding a fungal phytase which phytase catalyzes the liberation of inorganic phosphate from myoinositol hexakis-phosphate, which method comprises

(a) culturing the cells of claim 39 under conditions wherein said phytase-encoding nucleotide sequence is expressed to produce said phytase, and

(b) recovering said phytase produced from said culture.

44. (New) A method to identify nucleic acid molecules that encode compounds havig phytase activity which method comprises

subjecting a candidate nucleic acid molecule to an assay to assess its ability to hybridize with a probe selected from SEQ ID NO:25, SEQ ID NO:26, or SEQ ID NO:27, and

subjecting a compound encoded by a nucleic acid molecule that hybridizes to said probe to an assay that measures phytase activity, wherein a nucleic acid molecule that encodes a compound that has phytase activity is identified.

45. (New) The method defined in claim 44 wherein the assay that measures phytase activity comprises determining whether the compound encoded by a nucleic acid molecule catalyzes the liberation of inorganic phosphorous from myoinositol hexakis-phosphate

46. (New) The method defined in claim 44, wherein the nucleic acid molecule is obtained from a fungal source.

47. (New) A recombinant expression system which is useful, when contained in a host cell, for expressing a nucleic acid molecule encoding a phytase, the expression system

comprising a nucleic acid molecule identified according to the method of claim 44, or a nucleic acid molecule derived therefrom by degeneration of the genetic code, operably linked to control sequences compatible with the host cell.